Neuroscience Letters 479 (2010) 181–186

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Effects of *Eucommia* leaf extracts on autonomic nerves, body temperature, lipolysis, food intake, and body weight

Yuko Horii[a,*], Mamoru Tanida[a], Jiao Shen[a], Tetsuya Hirata[b], Naomi Kawamura[b], Atsunori Wada[b], Katsuya Nagai[a]

[a] *ANBAS Corporation, 4-12-17 Toyosaki, Kita-Ku, Osaka 531-0072, Japan*
[b] *R&D Center, Kobayashi Pharmaceutical Co., Ltd., 1-30-3 Toyokawa, Ibaraki, Osaka 567-0057, Japan*

ARTICLE INFO

Article history:
Received 23 July 2009
Received in revised form 30 April 2010
Accepted 11 May 2010

Keywords:
Brown adipose tissue
White adipose tissue
Stomach
Sympathetic nerve
Parasympathetic nerve
Obesity

ABSTRACT

Eucommia ulmoides Oliver leaf extracts (ELE) have been shown to exert a hypolipidemic effect in hamsters. Therefore, it was hypothesized that ELE might affect lipid metabolism via changes in autonomic nerve activities and causes changes in thermogenesis and body weight. We examined this hypothesis, and found that intraduodenal (ID) injection of ELE elevated epididymal white adipose tissue sympathetic nerve activity (WAT-SNA) and interscapular brown adipose tissue sympathetic nerve activity (BAT-SNA) in urethane-anesthetized rats and elevated the plasma concentration of free fatty acids (FFA) (a marker of lipolysis) and body temperature (BT) (a marker of thermogenesis) in conscious rats. Furthermore, it was observed that ID administration of ELE decreased gastric vagal nerve activity (GVNA) in urethane-anesthetized rats, and that ELE given as food reduced food intake, body and abdominal adipose tissue weights and decreased plasma triglyceride level. These findings suggest that ELE stimulates lipolysis and thermogenesis through elevations in WAT-SNA and BAT-SNA, respectively, suppresses appetite by inhibiting the activities of the parasympathetic nerves innervating the gastrointestinal tract, including GVNA, and decreases the amount of abdominal fat and body weight via these changes.

Eucommia ulmoides Oliver leaf extracts (ELE) have been suggested to have a hypolipidemic effect in hamsters [2] and rats [6]. Since BAT mainly uses fatty acids as fuel for thermogenesis [5], the excitation of the sympathetic nerves innervating BAT is thought to reduce the triglycerides (TGs) content in WAT. Therefore, we hypothesized that ELE might affect white adipose tissue sympathetic nerve activity (WAT-SNA) and brown adipose tissue sympathetic nerve activity (BAT-SNA). We examined the effects of intraduodenal (ID) administration of ELE on WAT-SNA and BAT-SNA in urethane-anesthetized rats, and found that ELE elevated WAT-SNA and BAT-SNA. We then examined the effects of ELE on the plasma FFA concentration, and BT above BAT in unanesthetized rats because excitation of WAT-SNA and BAT-SNA causes lipolysis and thermogenesis, respectively. On the other hand, parasympathetic nerves innervating the stomach play an important role in digestion and absorption. Therefore, we examined the effects of ID administration of ELE on gastric vagal nerve activity (GVNA), food intake, and body weight in rats.

Male Wistar rats, weighing 300–350 g, housed in a room maintained at 24 ± 1 °C and illuminated for 12 h (800–2000 h) were used. Food and water were freely available. The rats were adapted to this environment for at least 1 week before the experiment. All animal care and handling procedures were approved by the Animal Research Committee of the ANBAS Corporation.

Eucommia ulmoides Oliver leaves collected in Sichuan District of China were treated with steam at 100–110 °C, and then, dried and roasted. Two tons of roasted *Eucommia* leaves were extracted with 10 tons of hot water at 90 °C for 1 h, and the extract was filtered and concentrated. The concentrate was allowed to stand for a day. It was again filtered, concentrated, vacuum-dried, and powdered (yield: 18%).

The general procedure has been described previously [10]. Briefly, under anesthesia [1.2 g/kg urethane, intraperitoneal (IP)], a polyethylene catheter was inserted into the duodenum for ID injection. The sympathetic nerves innervating the left epididymal WAT and right interscapular BAT, and GVNA were ligated at their distal ends, and hooked to a pair of silver wire electrodes for recording efferent nerve activities. The rats were allowed to stabilize for 60 min after the recording electrodes were connected. Electrical changes in these autonomic nerves were amplified, filtered, and monitored using an oscilloscope. Data were obtained as described previously [10]. Baseline measurements of WAT-SNA, BAT-SNA, and GVNA were performed 5 min before ID injection of 1 ml aqueous solution of ELE (in 0.01–500 mg/ml water; Kobayashi Pharmaceutical Co., Ltd., Japan) or water.

* Corresponding author. Tel.: +81 6 6486 1080; fax: +81 6 6486 1081.
E-mail address: yhorii@anbas.co.jp (Y. Horii).

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.05.030

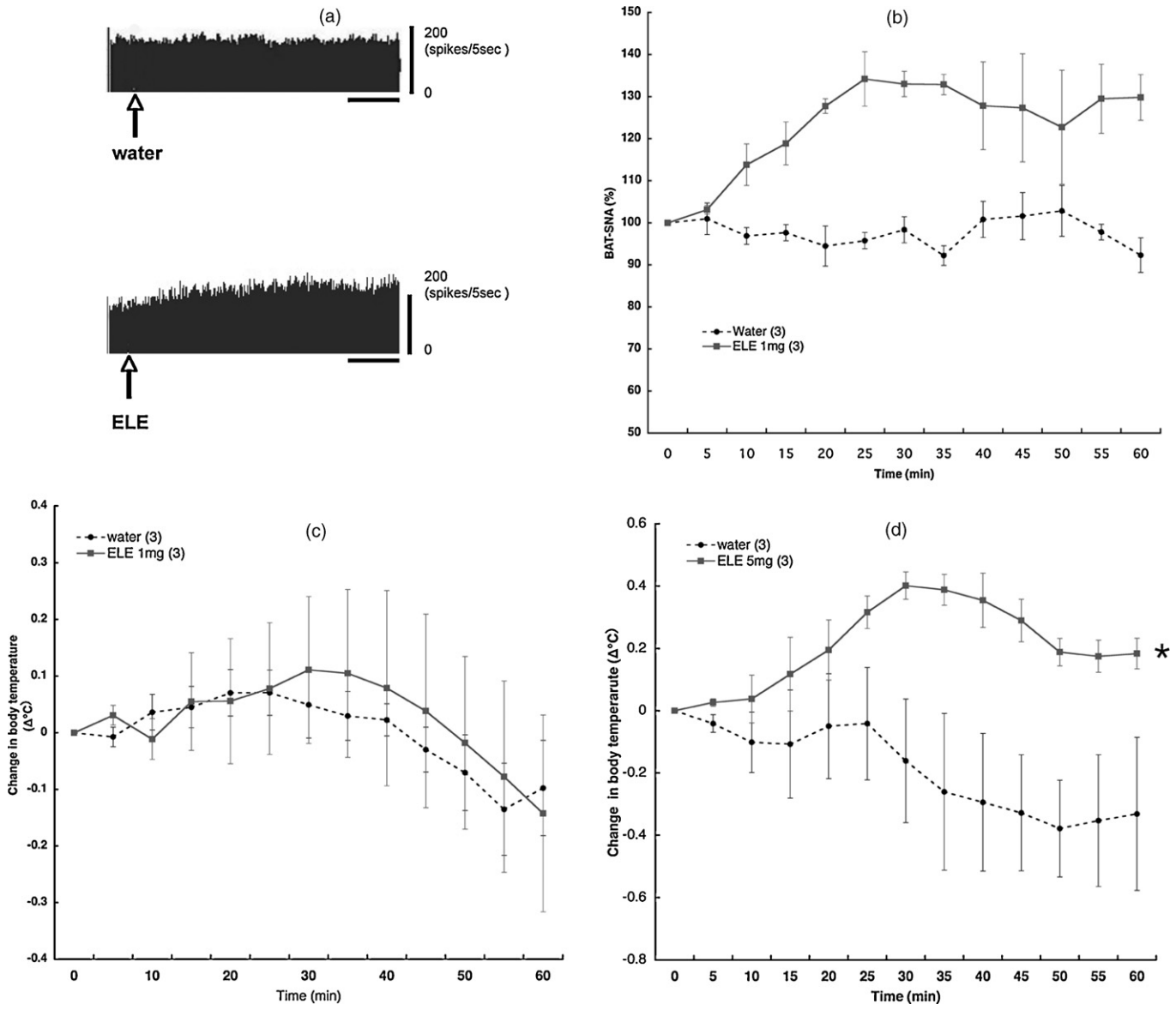

Fig. 1. Effects of intraduodenal (ID) injection of *Eucommia* leaf extracts (ELE) on interscapular brown adipose tissue sympathetic nerve activity (BAT-SNA) and the change in body temperature above the interscapular brown adipose tissue. (a) Typical recordings of BAT-SNA; horizontal bars represent 10 min. (b) Changes in BAT-SNA (%) injected with 1 ml aqueous solution of ELE (1 mg/ml) or water. The number of animals used is shown in parentheses. The significant difference between the values after injection of water and ELE was analyzed by ANOVA ($P < 0.05$). (c) Changes in body temperature (D°C) from basal values (values at 0 min) after ID injections of 1 mg ELE. (d) Changes in body temperature (D°C) from basal values (0 min) after ID injections of 5 mg ELE or water. The number of animals used is shown in parentheses. The significant differences between the values after injection of water and ELE were analyzed by ANOVA ($P < 0.05$).

A telemetry system (Star Medical Co., Tokyo) was used to measure BT as described previously [12]. Briefly, 2 days before the experiment, a capsule containing a temperature sensor was implanted into the subcutaneous space above the interscapular BAT, and an ID cannula was inserted under pentobarbital anesthesia (35 mg/kg, IP). On the experimental day, food was removed 4–6 h prior to ID injection of ELE or water. Baseline BT was measured without anesthesia 5 min before ID injection of ELE or water, which was given at approximately 1300 h. Analog output signals obtained by a receiver were converted to digital data, monitored, and stored in a computer. The data were analyzed using the 16ch-Eight Star program (Star Medical Co.).

The plasma FFA level was determined as described previously [8]. Briefly, a jugular vein catheter was inserted under pentobarbital anesthesia (35 mg/kg, IP) 3 days before the experiment and on the experimental day blood samples (0.3 ml each) were collected using the jugular vein catheter without anesthesia before (0 min) and 30, 60, 90, and 120 min after ID injection of ELE or water. Food was removed before the start of the experiment. The plasma FFA concentration was assayed using the NEFA-C-Test kit (WAKO Pure Chemical Industries, Ltd., Osaka) [8].

Ten rats were divided into 2 groups on the basis of body weights and given a control high-fat diet (HFD) [Oriental Yeast Co., Ltd, Tokyo; dietary composition (weight %): fat, 24.6% (beef tallow, 11.5%; lard, 11.5%; and soybean oil, 1.6%); casein, 22.6%; corn starch, 22.07%; sucrose, 20%; cellulose, 5.6%; mineral mixture (AIN-93G), 3.5%; vitamin mixture (AIN-93), 1%; choline bitartrate, 0.25%; tertiary butyl hydroquinone, 0.01%; and cystine, 0.38%; caloric density, 510.9 kcal/100 g] and a HFD containing 10% ELE [dietary composition (weight %): fat, 27% (beef tallow, 12.6%; lard, 12.6%; and soybean oil, 1.8%); casein, 22.5%; corn starch, 13.38%; sucrose, 18%; cellulose, 4.5%; mineral mixture (AIN-93G), 3.15%; vitamin mixture (AIN-93), 0.9%; choline bitartrate, 0.23%; tertiary butyl hydroquinone, 0.01%; cystine, 0.34%; and ELE, 10%; caloric density,